bio-protocol Preprint

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Neoangiogenesis

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An abbreviated version of this protocol was published in Science Advances in Oct 2020

Circadian disruption promotes tumor-immune microenvironment remodeling favoring tumor cell proliferation

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Detailed protocol

Neoangiogenesis protocol

- Cultivate mouse melanoma cell line B16F0 in complete Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientifc) supplemented with 5% (v/v) fetal bovine serum (FBS) and maintained at 37°C in a humidified incubator with 5% CO_2.
- Cell preparation: 100.000 B16F0 cells, or vehicle, with Trypan Blue (0.2%) in relation 1:1. Final volume: 50 µl.
- Inoculate intradermally in the dorsal cephalad site of mice.
- Seven days later, anesthetize each mouse with isoflorane inhalant and sacrifice them.
- Perform a longitudinal incision in the medial ventral line and open the skin of the inoculation site (take special care to not cut the inoculation site).
- Put a 1.5 cm x 1.5 cm side squad centered on the injection site (identified as a blue point) and take pictures.
- Count blood vessel bifurcations (each new branch) from the pictures using ImageJ software (National Institutes of Health, Maryland, USA, https://imagej.nih.gov/ij/).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Golombek, D. and Paladino, N. (2020). Neoangiogenesis. Bio-protocol Preprint. bio-protocol.org/prep577.
2. Aiello, I., Fedele, M. L. M., Román, F., Marpegan, L., Caldart, C., Chiesa, J. J., Golombek, D. A., Finkielstein, C. V. and Paladino, N.(2020). Circadian disruption promotes tumor-immune microenvironment remodeling favoring tumor cell proliferation. Science Advances 6(42). DOI: 10.1126/sciadv.aaz4530

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